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α-Tocopherol and Polyunsaturated Fatty Acid Membrane Domians

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While α-tocopherol's role as the major membrane antioxidant has been certain for decades, its membrane structural role is far less certain. We are interested in a possible involvement of α-tocopherol in helping to hold proposed polyunsaturated fatty acid (PUFA)-rich, non-raft, domains together. The experiments presented here to test this hypothesis include differential scanning calorimetry (DSC), cold temperature detergent extractions and isothermal titration calorimetry (ITC) studies on model bilayer membranes and detergent extractions on living cell membranes. The DSC experiments indicate that in model membranes composed of sphingomyelin (SM) and 1-palmtioyl-2-docosahexaenoylphosphatidylethanolamine (16:0-22:6PE), cholesterol has a stronger association with SM while α-tocopherol prefers the polyunsaturated PE. Triton X-100 extractions on the same model system confirm that cholesterol segregates with SM (detergent insoluble fraction) and α-tocopherol segregates with 16:0-22:6PE (detergent soluble fraction). Currently underway are ITC experiments that measure the partitioning of α-tocopherol between lipid vesicles to quantify the stronger affinity for polyunsaturated lipids, and detergent extractions on living lymphocytes, where once again cholesterol is shown to preferentially segregate with SM into raft fractions, to observe preferential segregation of α-tocopherol with PUFA into non-raft fractions.

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Membrane Organization of Vitamin E is Sensitive to Lipid Unsaturation

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Vitamin E (α-tocopherol) has long been known as the major antioxidant in biological membranes. However, there remain many structurally related questions. Details of the molecular organization of α-tocopherol in membranes, for instance, lack the precision to address whether the vitamin has preferential affinity for unsaturated lipids in support of its role as an antioxidant. To observe how α-tocopherol interacts with unsaturated phospholipids, we determine, from one-dimensional neutron scattering length density profiles, the depth of deuterated analogs in phosphatidylcholine (PC) bilayers. The profiles obtained with α-[5-$^2H_3$]tocopherol and α-[9′-$^2H_2$]tocopherol in 1,2-dioloeylphosphatidylcholine (18:1-18:1PC) bilayers place the centers of mass of the labels 13 and 0 Å, respectively, from the bilayer center. They are consistent with the vitamin molecule sitting upright in the bilayer so that the hydroxyl group on the chromanol is near the aqueous interface and a highly disordered sidechain extends towards the middle of the membrane. The profile obtained for α-[5-$^2H_3$]tocopherol in 1-palmitoyl-2-oleeylphosphatidylcholine (16:0-18:1PC) reveals that, in contrast, the center of mass of the label sits 10 Å higher than in 18:1-18:1PC. A remarkable sensitivity upon membrane unsaturation for the depth of penetration of vitamin E is implied, and we are currently using solid state ^2H NMR and MD simulations to provide a detailed view of dynamical organization.

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Perturbation of Membrane Structure by Oxysterols

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Cholesterol is essential to the regulation and function of cell membranes, and cells expend large amounts of energy to control membrane cholesterol levels. The oxysterols, oxidation products of cholesterol, are enzymatically produced molecules that play a major role in regulating cholesterol homeostasis. Recent experimental work has shown that 25-hydroxycholesterol can affect cholesterol homeostasis through non-enantioselective mechanisms. Using molecular dynamics simulations, we have shown that cholesterol and 25-hydroxycholesterol alter membrane properties in very different ways, and that these effects are rooted in their orientations within the membrane. Newer simulations of bilayers containing both cholesterol and 25-hydroxycholesterol in the same membrane have shown that the presence of 25-hydroxycholesterol alters the position and orientation of cholesterol, increasing its solvent accessibility. Our work suggests that cholesterol and membrane perturbation by oxysterols may play a role in the oxysterol regulation of cholesterol homeostasis.

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Dynamics of Sedimentation and Deformation of GUVs Under Different Tonicity Conditions

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POPC Giant unilamellar vesicles (GUVs) with 1 mol% DiIC18(3) as a fluorescent marker were prepared by electroformation in sucrose solutions with varying osmolarities. These vesicles were resuspended in glucose solutions of different concentrations generating isotonic and hypertonic conditions. Vesicles sediment due to the density difference between the solutions. The movement of the vesicles as they approach the glass surface is studied using SPIM microscopy. We find that the velocity of the GUVs remains constant, given by Stokes law as expected when the distance from the surface is several radii in length. Velocity decreases exponentially as vesicles reach the surface. Vesicle deformation due to interactions with the surface was measured for different osmotic conditions using confocal and SPIM microscopy.

Boundary element simulations were performed to model vesicle deformation during sedimentation within a viscous fluid. For isotonic conditions, vesicles are assumed to begin with zero tension and tension is generated through contact with the surface. For the hypertonic case, the same is true but with an initial excess area available. Computationally, the mechanical behavior of the lipid bilayer is simulated using a model that considers two modes of deformation responsible for increases in area strain. The first is the smoothing of sub-optical thermal undulations and the second is the direct stretching of the area per lipid molecule. Properties of the lipid bilayer are controlled by adjusting bending and area compressibility moduli. A force field is implemented that takes into account local tension, local curvature force, and gravitational pull. Vesicle sedimentation, deformation, and membrane tension were evaluated as a function of g0, a dimensionless factor relating gravitational and curvature energies. Simulations are in agreement with the experimental results and provide additional information of the deformation of vesicles and sedimentation dynamics.

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Structure and Phase Behavior of Cholesterol Containing Membranes in the Presence of Ethanol

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Molecular dynamics (MD) of cholesterol containing membranes is used to examine the structural changes and phase behavior of lipids in the presence of varying ethanol concentrations over a range of temperatures. Alcohols are known to cause changes in the phase transitions of phospholipids as well as inducing the formation of an interdigitated phase of reduced thickness, where the hydrophobic tails of the top and bottom lipids intercalate causing an increase in the area per lipid as well as the solvent exposed surface of the headgroups. Atomistic MD simulations using the Gromacs 4.0 software allows analysis of structural changes in lipid volume, area per lipid, and hydrogen bonding among others at the molecular level. Pure 1,2-Dioleoyl-sn-Glycero-3-Phosphocholine (DOPC) and DOPC/cholesterol membranes were constructed to have 128 lipid molecules under full hydration. DOPC/cholesterol systems contain 10, 20, and 30 mole % cholesterol, and those containing ethanol had additional 5, 10, 15 and 20 % V/V ethanol molecules in the solvent. All systems were simulated at 6 different temperatures which span relevant biological processes for biofuel production; also experimentally observed phase changes occur at some of these temperatures. The effects of ethanol on biological membranes are of considerable importance to the studies of biofuels, enology, and medicine.

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Miscibility Phase Behavior of GUV Membranes Containing Charge: Ternary Mixtures of Cholesterol, PC-Lipids, and PG-Lipids

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Giant unilamellar vesicles composed of a ternary mixture of phospholipids and cholesterol exhibit coexisting liquid phases over a range of temperatures and compositions. Few studies of phase behavior have been made using charged lipids, even though they account for a significant fraction of lipids in biological membranes. Here, I present phase diagrams of vesicles